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Ultra-sensitive detection of IgE using biofunctionalized nanoparticle-enhanced SPR

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ABSTRACT

This paper describes an ultra-sensitive surface-based detection method using nanoparticle-enhanced surface plasmon resonance (SPR) for the detection of immunoglobulin E (IgE) proteins, which could potentially be used for the diagnosis of allergic diseases. Two different probes, anti-IgE and IgE specific aptamers, which can specifically interact with IgE at different epitopes were first investigated for their specific interaction with IgE using SPR. Langmuir adsorption coefficient (K_{ads}) values were measured as $2.0(\pm0.22) \times 10^8\ M^{-1}$ and $2.2(\pm0.20) \times 10^8\ M^{-1}$ for IgE interactions with anti-IgE and IgE specific aptamers, respectively. The SPR detection limit of the simple adsorption of IgE onto either anti-IgE or IgE specific aptamers was found to be about 1 nM. In order to improve the SPR detection signal for IgE, two different approaches utilizing surface formed sandwich complexes with biofunctionalized gold nanoparticles (Au-Nps) were designed and their detection performance were compared; the complexes were created via the adsorption of IgE onto (i) surface immobilized anti-IgE followed by the adsorption of IgE specific aptamer coated gold nanoparticles and (ii) IgE specific aptamer surface with the subsequent adsorption of anti-IgE coated gold nanoparticles. Both detection schemes were able to directly measure IgE at femtomolar concentrations.

1. Introduction

An allergic reaction can result in various autoimmune diseases including atopic dermatitis, bronchial asthma, plant allergy, urticaria, and eczema [1]. In particular, atopic dermatitis is of great interest since patient numbers have dramatically increased in recent years with over 10% of children worldwide thought to be suffering from an atopic related allergy [2]. Dermatitis symptoms occur indiscreetly on the skin anywhere on the body and are often accompanied with severe fever, asthma and conjunctivitis. Atopic illnesses are associated with inappropriate stimulation of immunoglobulin E (IgE) production by an allergic reaction to substances such as drugs, chemical materials, pollen, a vegetable fiber, bacteria, foods and hair colorings [3]. The facile measurement of human IgE protein levels could thus be usefully employed for the diagnosis of atopic dermatitis as well as other human allergic diseases [1].

A wide range of detection methodologies have been developed for the detection of IgE including fluorescence microarray technology [4,5], matrix-assisted laser desorption ionization (MALDI-TOF) [6,7], atomic force microscopy (AFM) [8,9], quartz crystal microbalance (QCM) [10] in addition to conventional chromogenic or fluorescence enzyme immunoassays [11,12]. For example, Weller et al., demonstrated antibody microarrays created on a glass substrate were successfully utilized to screen allergen-specific IgE in human serum samples via the detection of fluorescently labeled secondary antibodies interacting with the surface formed IgE/anti-IgE complex [5]. Aptamers specific to IgE have also been applied in surface-based detection techniques such as MALDI-TOF [7] and AFM [9] with the detection of pM levels of IgE reported. Both QCM [10] and electrochemical based approaches [13] have been demonstrated at nM detection levels for IgE using either anti-IgE or IgE specific aptamers immobilized on gold surfaces.

The direct detection of IgE could also be achieved using a surface sensitive label-free detection method such as SPR which measures reflectivity changes due to the interaction of biomolecules (e.g., IgE) from a biological sample solution with target specific probe molecules attached onto the surface of a functionalized thin gold metal film. Recently, Chen et al., has utilized SPR to detect nM concentrations of IgE utilizing aptamer probes [14]. Further improving the detection limit of SPR to pico- and femtomolar target concentrations has resulted in a variety of novel

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amplification strategies. For example, surface enzyme reactions were utilized in conjunction with RNA microarrays created on gold surfaces for the detection of 10 fM genomic DNA [15]. In addition, biomolecule-conjugated nanoparticles including either DNA- or antibody-coated nanoparticles have been utilized to enhance SPR detection signals [16–20]. Moreover, the combined use of enzymatic amplification and biofunctionalized nanoparticles were incorporated into the SPR detection scheme for the measurements of microRNAs down to 10 fM [17].

In this paper, we demonstrate a novel surface-based detection method using biofunctionalized nanoparticles with SPR for the ultra-sensitive detection of a protein biomarker, IgE. The protein has two different binding sites for anti-IgE and IgE specific aptamers introducing options for either to be immobilized as a probe on the SPR chip surface or conjugated to a nanoparticle. This allows the design of two different sandwich assay detection schemes to directly detect IgE at femtomolar concentrations without the need for labeling. We first investigated the specific interaction of both anti-IgE and IgE specific aptamers with IgE using real-time SPR. Improvement in preventing non-specific binding of proteins onto the chip surface was studied by creating mixed monolayers of ω-functionalized alkane-thiol and different molecular weights of polyethylene glycol (PEG) molecules. Next, two different ultra-sensitive detection approaches utilizing sandwich assay complexes with biofunctionalized gold nanoparticles (Au-Nps) were designed and their detection capabilities for IgE were compared. Two sandwich assay complexes were created via the adsorption of IgE onto (i) surface bound anti-IgE followed by the adsorption of IgE-aptamer coated gold nanoparticles and (ii) IgE-aptamer surface before the adsorption of anti-IgE coated nanoparticles.

2. Experimental

2.1. Chemicals

11-Mercaptoundecanoic acid (MUA; Aldrich), 11-amino-1-undecanethiol hydrochloride (MUAM; Dojindo), 1,1-carbonyldiimidazole (CDI; Aldrich), sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SSMCC; Pierce), triethanolamine (TEA; Sigma), thiol-modified polyethylene glycol, MW 180, 232, 1500 (PEG-SH; Paraon), human immunoglobulin E (IgE, 190 kDa; Calbiochem), goat anti-human immunoglobulin E, ε-chain specific, (anti-IgE, 150 kDa; Calbiochem), bovine serum albumin (BSA, 66 kDa; Calbiochem), bovine serum albumin antibody produced in rabbit (anti-BSA, 155 kDa; Aldrich) were all used as received. All rinsing steps were performed using absolute ethanol or acetone or Millipore filtered water. IgE specific DNA aptamer and control DNA were custom-synthesized by Integrated DNA Technologies; IgE-aptamer sequence is 5′-thiol-AAA AAA AAA AAA AAA GGG GCA CGT TTA TCC GTC CCT CCT AGT GGC GTG CCC G-3′ (IgE-SH; IDT) and control DNA is 5′-thiol-AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA-3′ (A_{30}-SH; IDT). All biomolecules were buffered in phosphate saline buffer (PBS, pH 7.4; Invitrogen). All experiments were performed at room temperature unless otherwise stated.

2.2. Surface attachment of anti-IgE onto a gold thin film

For the antibody immobilization, a bare gold chip from Biacore (45 nm thick) was soaked in a mixed solution of 0.5 mM MUA and 0.05 mM PEG-SH for 12 h. The gold chip was then immersed into a solution of CDI dissolved in acetone (10 mg/mL) overnight to form an amine-reactive imidazole surface followed by exposure to a 10 nM of anti-IgE solution for 30 min [21]. The gold chip was then rinsed with Millipore water and dried under a nitrogen stream prior to use.

2.3. Surface immobilization of IgE-aptamer onto a gold thin film

The gold chip functionalized with a mixed monolayer of 0.5 mM MUAM and 0.05 mM SH-PEG was reacted with a 1 mM solution of SSMCC in 0.1 M TEA (pH 7) buffer for 30 min to form a thiol-reactive maleimide-terminated surface [18]. Next, the chip was exposed to a 1 mM solution of 5′-thiol-modified IgE specific aptamer overnight followed by rinsing with Millipore water and dried under a nitrogen stream prior to use.

2.4. Biofuntionalization of gold nanoparticles

Gold nanoparticle colloids having an average particle diameter of 13 ± 1 nm with a λ_{max} of 519 nm were first synthesized using a standard citrate reduction method described by Natan and coworkers [22]. Conjugation of antibodies to Au nanoparticles was then performed using a modified procedure described by Long and Keating [23]. Briefly, 100 μL of 40 mM sodium phosphate buffer (pH 7.4), 100 μL of 4 μM anti-IgE and 100 μL of 1.0 M NaCl were added to 800 μL of Au nanoparticle solution in an eppendorf tube. The solution was then gently shaken for several seconds and kept at 4 °C for 1 h to immobilize antibodies onto nanoparticles. Next, the solution was centrifuged (10 min at 14,700 rpm) to separate the unbound antibody from Au nanoparticle–antibody conjugates. The conjugate was then resuspended to 1 mL with phosphate buffered saline (PBS), pH 7.4 and the conjugation (λ_{max} of 524 nm) was confirmed by a small 5 nm red shift in the resonance peak compared to that of the uncoated nanoparticles using UV–vis spectroscopy. DNA aptamer coated Au nanoparticles were prepared using a protocol reported by Li et al. [18]; 50 μL of 100 μM 5′-thiol-modified IgE specific aptamer was mixed with 800 μL of the gold nanoparticle solution followed by the addition of 230 μL of H_2O and 120 μL of 1 M NaCl/100 mM phosphate (pH 7.4) buffer. The mixed solution was stored at 37 °C for 4 h and centrifuged for 40 min at 12,000 rpm to remove unbound aptamers from Au–aptamer conjugates. The conjugation solution was finally resuspended in PBS (pH 7.4) solution and the formation of conjugates (λ_{max} = 526 nm) was verified via a ca. 7 nm peak red shift compared to the original nanoparticle solution (λ_{max} = 519 nm) using UV–vis spectroscopy.

2.5. SPR measurements

A four-channel Biacore SPR system (Biacore 3000) was utilized for all real-time monitoring of target biomolecules interacting with surface immobilized probe molecules. In order to account for possible contributions to the SPR signal due to nonspecific adsorption of non-target, two channels were used to inject target samples and the remaining two channels were used to introduce either reference or negative control probe samples. PBS (pH 7.4) buffer and a flow rate of 5 μL/min were used unless otherwise stated. Surface immobilized anti-IgE and IgE specific aptamers were regenerated using 0.05 M HCl and 8 M urea, respectively for minimum 40 min before the injection of different concentrations of target proteins.

3. Results and discussion

3.1. Interaction of IgE with surface immobilized anti-IgE and IgE-aptamers

In order to utilize SPR for the quantitative analysis of proteins, it is important to distinguish specific and non-specific adsorption of target proteins onto surface immobilized probe molecules. Treatment of surfaces with blocking agents such as polyethylene glycol (PEG), BSA or protein A [24–26] in addition to a controllable and robust attachment of probe molecules on surfaces can improve the performance of surface-based detection methods. Before studying

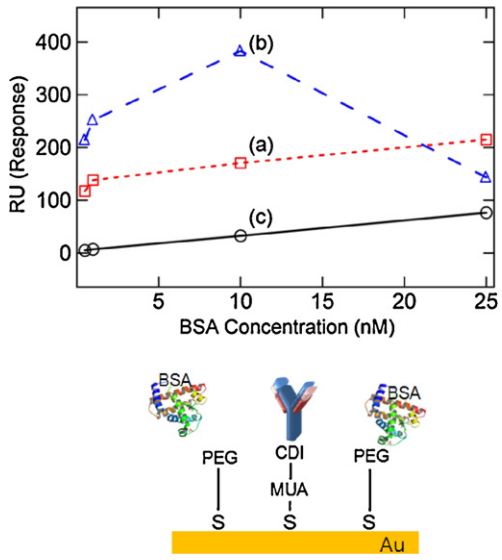

Fig. 1. Plots summarizing SPR responses for the adsorption of various concentrations of BSA onto anti-IgE probes immobilized on a mixed monolayer of MUA and different molecular weights of PEG-SH. Molecular weights of PEG molecules were (a) 180, (b) 230 and (c) 1500 and BSA concentrations were varied from 1 nM to 25 nM.

the specific interaction of IgE with two different probing ligands, anti-IgE and IgE specific aptamers, which have different binding sites on IgE, the gold surface was modified with a mixed monolayer of thiol-modified polyethylene glycol (PEG-SH) and ω-functionalized alkane-thiol followed by the covalent attachment of probe molecules using a cross-linker such as CDI for antibodies and SSMCC for 5′-thiol-modified aptamers. Three different sizes of PEG-SH molecules (MW 180, 230 and 1500) were investigated as part of the mixed monolayer to determine which is the most effective at reducing non-specific interactions between non-target proteins and the anti-IgE functionalized sensor surface.

Fig. 1 shows the effects of non-specific adsorption of BSA onto an anti-IgE prepared surface as a function of BSA concentration. PEG (MW 1500) molecules were found to be the most effective at reducing non-specific binding of BSA compared to that of smaller MW PEG molecules. In addition, analysis of SPR measurements (data not shown) acquired for the specific interaction of IgE comparing the different mixed monolayer compositions showed the highest signal for PEG (MW 1500). An alternative option was also investigated involving treating the sensor surface with BSA after IgE immobilization, however changes in the SPR signal associated with non-specific interaction of protein A were significant for all monolayer compositions with values close to that of the PEG (MW180) data in Fig. 1. Consequently, all subsequent work was performed on a mixed monolayer of PEG (MW 1500) and ω-functionalized alkane-thiol to which either anti-IgE or IgE specific aptamers were covalently coupled.

The binding strength of IgE with anti-IgE and IgE specific aptamers immobilized on the mixed monolayer was next investigated. Fig. 2 shows representative real-time SPR sensorgrams obtained for the detection of 25 nM IgE using surface immobilized (a) anti-IgE and (b) IgE specific aptamer. Various IgE concentrations ranging from 0.5 nM to 100 nM were injected and about 1 nM of IgE was easily detected with both probe molecules. The differences in SPR signals (RU) before and after IgE adsorption were then used to obtain Langmuir adsorption coefficient values.

Fig. 3 shows plots of surface coverage (θ) of IgE binding onto (a) anti-IgE and (b) IgE specific aptamer as a function of IgE concentration. Assuming that IgE interacts with either anti-IgE or IgE-aptamer with the reaction ratio of 1:1, the data in Fig. 3 can

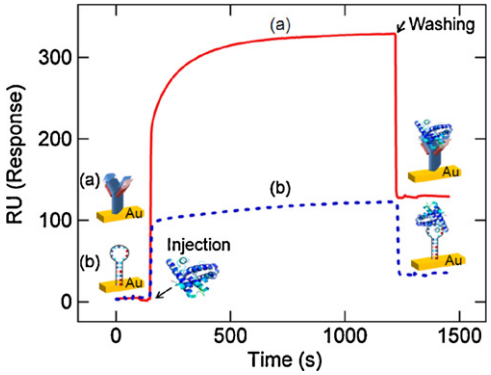

Fig. 2. Representative real-time SPR responses (RU) for monitoring the adsorption of 25 nM IgE onto (a) anti-IgE chips and (b) IgE-aptamer chips.

be fitted using the Langmuir isotherm equation:

$$\theta = \frac{K_{ads}C}{1 + K_{ads}C} \quad (1)$$

where C is the IgE concentration and θ is the surface coverage obtained by normalizing SPR (RU) signals with the maximum binding of IgE onto probe molecules (maximum SPR signal) is almost one. From the fit, Langmuir adsorption coefficients ($K_{ads} = k_a/k_d$) of $2.0(\pm 0.22) \times 10^8\ M^{-1}$ and $2.2(\pm 0.20) \times 10^8\ M^{-1}$ were obtained for anti-IgE and IgE specific aptamer, respectively. The binding strengths of anti-IgE *versus* IgE-aptamer were found to be almost the same within the experimental error. This represents at least an order of magnitude stronger compared to previous literature values where anti-IgE and IgE-aptamer binding affinities were reported as $7.73 \times 10^6\ M^{-1}$ and $1.34 \times 10^7\ M^{-1}$, respectively [14]. This could mainly arise from differences in the surface chemistries used; our experiments utilized a mixed self assembled monolayer of PEG and longer alkane molecules, while the literature values were obtained using a self assembled monolayer of a shorter length of alkane-thiol, 3-mercapto-1-propanol, as a blocking agent. The use of longer alkane chain monolayers may act as a spacer to improve the acces-

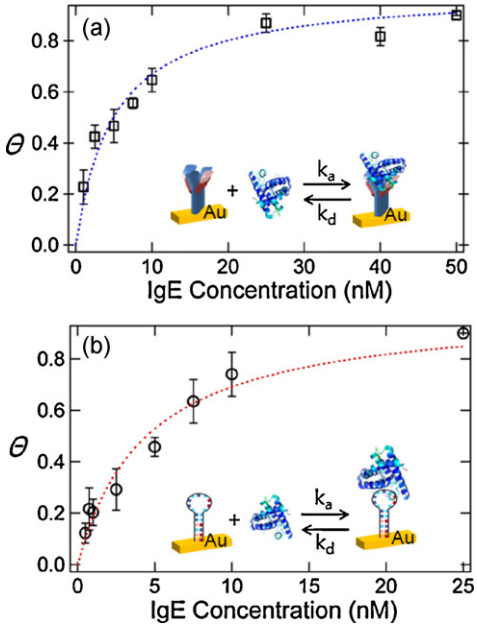

Fig. 3. Langmuir isotherm fitting of plots of surface coverage versus IgE concentration for (a) anti-IgE and (b) IgE aptamer surface immobilized probes. Concentrations of IgE ranged from 1 nM to 50 nM for the anti-IgE chip and from 0.5 nM to 25 nM for the IgE-aptamer chip.

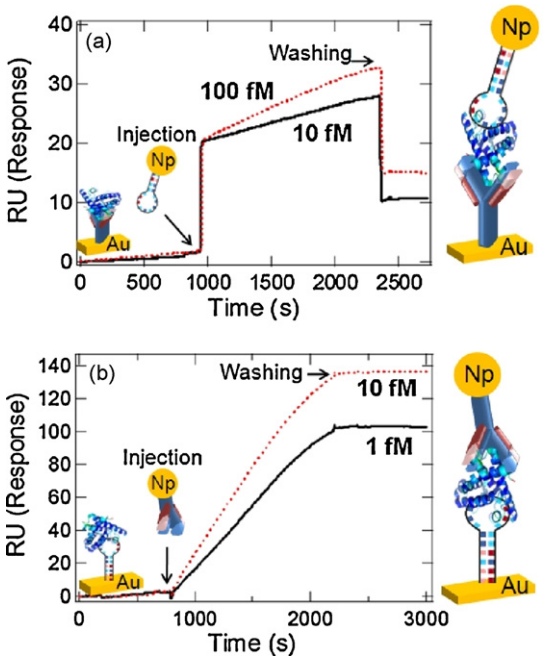

Fig. 4. A series of representative SPR sensorgrams for the detection of IgE using two different sandwich complexes formed on gold surfaces. (a) Adsorption of 100 fM (dotted line) and 10 fM (bold line) IgE onto surface immobilized anti-IgE followed by the binding of IgE-aptamer coated Au-Nps. (b) Adsorption of anti-IgE coated Au-Nps onto 10 fM (dotted line) and 1 fM (bold line) IgE complexed with surface bound IgE-aptamers.

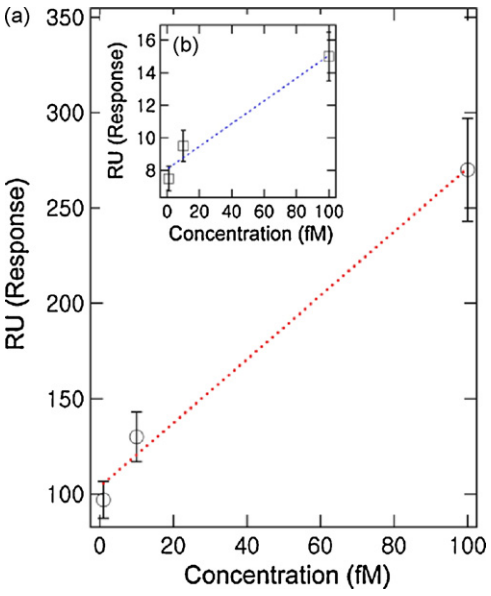

Fig. 5. Plots of the gold nanoparticle-enhanced SPR response obtained for IgE concentrations ranging from 1 fM to 100 fM. The dotted line in the figure is the linear fit of the data. (a) Adsorption of anti-IgE coated Au-Nps onto surface bound IgE/IgE aptamer complexes and (b) adsorption of IgE onto surface immobilized anti-IgE followed by the binding of IgE aptamer coated Au-Nps.

sibility of probes interacting with target molecules as well as the PEG molecules being a good blocking agent to reduce non-specific interaction between proteins and probes [25].

3.2. Ultra-sensitive SPR detection of IgE with biofunctionalized Au-Nps

Biofunctionalized gold nanoparticles (Au-Nps) have previously been employed in SPR measurements to effectively amplify the detection signal thus resulting in a great improvement in the detection limit [18]. Herein, we have designed two different detection schemes for the measurement of IgE utilizing two different sandwich complexes formed with biofunctionalized gold nanoparticles; (i) the adsorption of IgE onto surface bound anti-IgE followed by the adsorption of IgE-aptamer coated Au-Nps and (ii) the adsorption of anti-IgE coated Au-Nps onto the surface complex formed with IgE and surface immobilized IgE-aptamers.

Fig. 4 shows representative real-time SPR sensorgrams for the ultra-sensitive detection of IgE using (a) surface immobilized anti-IgE with IgE-aptamers coated Au-Nps and (b) surface bound IgE-aptamers with anti-IgE coated Au-Nps. IgE concentrations were varied from 1 fM to 100 fM. The specific adsorption of unlabeled IgE onto the sensor surface was first allowed to proceed for at least 1 h. The surface was then washed with PBS buffer for a minimum of 15 min to remove non-specific adsorption of IgE from the surface. The subsequent adsorption of IgE-aptamer coated Au-Nps onto the surface bound IgE /anti-IgE complex was continuously monitored till the SPR signal remained constant followed by washing the surface with buffer for 20 min. The same reaction procedure was employed with surface immobilized IgE aptamer probes.

To address the possibility of non-specific adsorption of gold nanoparticles onto either the aptamer or antibody surfaces, the SPR signal for the adsorption of anti-IgE coated Nps onto the IgE/anti-IgE complex was corrected using control data obtained for the adsorption of Nps coated with non-interacting antibody (i.e. anti-BSA) onto the surface IgE/anti-IgE complex in the reference channel. Similarly, the SPR signal change when using IgE-aptamer coated-Nps were corrected with respect to the signal obtained for the adsorption of Nps coated with non-interacting DNA (control DNA) sequences. In both cases the control signals were smaller at all target concentrations extending from the detection limit to higher.

As can be seen from Fig. 4, the SPR signal significantly increased for both detection schemes when biomolecule-conjugated nanoparticle probes were injected offering a detection capability in the region of 10 fM or better. In addition, plots of the corrected SPR signal with respect to the reference nanoparticles as a function of IgE concentrations are presented in Fig. 5. A linear relationship between IgE concentration and SPR response was obtained with slopes of 1.7 ± 0.2 and 0.07 ± 0.01 RU/fM for anti-IgE coated nanoparticles and IgE-aptamer coated nanoparticles, respectively. The detection capability using the sandwich formation of anti-IgE coated Au-Nps/IgE/surface bound IgE-aptamer was found to be about 10 times better than that involving the formation of IgE-aptamer Au-Nps/IgE/surface immobilized anti-IgE complexes. At higher IgE concentrations ranging from 500 fM to 100 pM, a reduced nanoparticle concentration could be utilized to re-establish the linear response of the SPR signal. Overall, both sandwich detection schemes demonstrate approximately 10^6 times enhancement in the detection limit compared to the simple binding of IgE only onto either surface immobilized IgE-aptamers or anti-IgE. Chip-to-chip variation was also investigated for both detection schemes using five different IgE-aptamer and anti-IgE chips with a ±10% variation in SPR signal observed for the injection of the same concentrations of both IgE and biomolecule-conjugated nanoparticles.

4. Conclusions

We have demonstrated two different sandwich detection schemes utilizing biofunctionalized nanoparticles in conjunction with SPR for the ultra-sensitive detection of a protein biomarker, IgE, which could be used for the diagnosis of allergic diseases. Au nanoparticle-enhanced sandwich detection methods capable of

measuring IgE down to 1 fM was demonstrated without any further labeling of the target protein itself. This is a remarkable 10^6 improvement upon conventional SPR measurements. In addition, our methods offer about 10^3 times better sensitivity compared to previous literature reports utilizing biofunctionalized nanoparticles to enhance SPR detection [18,19]. The excellent improvement in IgE detection sensitivity originates not only the use of a sandwich assay detection platform but also through the use of an optimized mixed monolayer of PEG and alkane-thiol molecules on the gold surface resulting in reduction of non-specific interactions.

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